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DATASET BRIEF

Proteomic study of the exponential-stationary growth phase transition in the haloarchaea *Natrialba magadii* and *Haloferax volcanii*

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Abbreviations

EXP, exponential; ST, stationary; TMS, transmembrane segment

Keywords

Comparative proteomics, Haloarchaea, *Haloferax volcanii, Natrialba magadii,* Stationary Phase

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Abstract

The dynamic changes that take place along the phases of microbial growth (lag, exponential, stationary and death) have been widely studied in bacteria at the molecular and cellular levels, but little is known for archaea. In this study, a high throughput approach was used to analyze and

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compare the proteomes of two haloarchaea during exponential and stationary growth: the neutrophilic *Haloferax volcanii* and the alkaliphilic *Natrialba magadii*. Almost 2000 proteins were identified in each species (~ 50% of the predicted proteome). Among them, 532 and 432 were found to be differential between growth phases in *H. volcanii* and *N. magadii*, respectively. Changes upon entrance into stationary phase included an overall increase in proteins involved in the transport of small molecules and ions, stress response and fatty acid catabolism. Proteins related to genetic processes and cell division showed a notorious decrease in amount. The data reported in this study not only contributes to our understanding of the exponential-stationary growth phase transition in extremophilic archaea but also provides the first comprehensive analysis of the proteome composition of *N. magadii*. The MS proteomics data have been deposited in the ProteomeXchange Consortium with the dataset identifier JPST000395.

Archaea constitute one of the three domains of life.[1] Many archaea grow optimally in extreme environments, meaning that they have developed biochemical and physiological adaptations. Haloarchaea thrive in hypersaline lakes and solar salterns containing > 2 M NaCl. They consist of neutrophilic (e.g. *Haloferax volcanii*, optimal growth at 2.5 M NaCl pH 7) as well as alkaliphilic species (e.g. *Natrialba magadii*, optimal growth at 3.5 M NaCl pH 9–10). Considering their extremophilic nature, archaea and their biomolecules represent a valuable resource for basic research as well as biotechnology, which still remains underexploited.[2, 3]

The whole proteome composition of several neutrophilic haloarchaea has been reported. This includes the high-throughput proteome characterization of *H. volcanii* parental strain and protease mutants like the proteasome,[4] the LonB[5] and the RhoII proteases;[6] *Haloarcula marismortui* during mid-log and late-log phases[7] and *Halobacterium salinarum* under different salt concentrations.[8] Meanwhile, in the haloalkaliphilic group only the cytoplasmic proteome of one member (*Natronomonas pharaonis*) has been examined.[9] Particularly in *N. magadii*, which has been a model organism for the study of proteases,[10-12] haloarchaeal viruses[13] and survival under space

conditions (high vacuum and UV radiation),[14] was analyzed using only comparative genomics methods.[15]

During the stationary phase, prokaryotes undergo several morphological and physiological changes that increase their stress resistance and allow them to survive under severe nutrient restrictions. These include the development of smaller and spherical cells, a rigid cell envelope, nucleoid condensation, a reduction in protein synthesis and an increase in peptidases/proteases[16]. Very few studies have explored the transition to stationary phase at a global scale in *Archaea*[17-19] and to the best of our knowledge, only two have done it at the proteome level: in the thermophile *Thermococcus kodakarensis* [20] and *H. marismortui*.[7]

We report the label-free shotgun proteomic analysis of two physiologically distinct haloarchaea (*H. volcanii* and *N. magadii*) during the logarithmic-stationary growth phase transition. Cultures of *H. volcanii* H26 and *N. magadii* ATCC 43099 were grown at 42 °C 200 rpm in rich medium (MGM and Tindall medium, respectively).[21, 22] Three independent cultures (biological replicates) of each organism were analyzed and samples were taken at exponential (EXP) (OD_{600} ~ 0.5) and stationary (ST) (OD_{600} ~ 1.5) growth phases. *H. volcanii* membrane and cytoplasm fractions were obtained as described previously.[6] *N. magadii* cells were disrupted by sonication in presence of 2 mM EDTA and 1 mM PMSF. The clarified cell extract supernatants were precipitated with 1 volume of acetone (O/N, 4 °C) and washed with acetone three times. Protein samples were processed, digested with trypsin and subjected to LC-ESI-MS/MS using a nanoACQUITY gradient UPLC pump system (Waters) and an LTQ Orbitrap Elite mass spectrometer as described in Cerletti et al.[5]

Proteins were identified and quantified with MaxQuant version 1.5.3.17[23] using the LFQ algorithm searching against the complete proteome database of *H. volcanii DS2* (4035 entries, September 2013) and *N. magadii* ATCC 43099 (4023 entries, January 2017) exported from the Halolex database.[24] The parameters were set as follows: main search peptide mass tolerance of 4.5 ppm, min. peptide length of 6 amino acids with max. two missed cleavages, routine posttranslational

modifications were searched including variable oxidation of methionine, deamidation (NQ), N-terminal glu->pyroglutamate and protein N-terminal acetylation, LFQ min. ratio count of 2, matching between runs enabled, PSM and (Razor) protein FDR of 0.01, advanced ratio estimation and second peptides enabled. FDR was set to 0.05 using the permutation of data between samples implemented in Perseus[25] (250 permutations, S0=0.1). Proteins were considered as identified if they were detected at least in one replicate. A student's t-test was performed to determine significantly regulated proteins. A log2 value of at least 1 and -1 (2 fold change) and an FDR (q-value) < 0.05 were used as cutoff. The MS proteomics data have been deposited to the ProteomeXchange Consortium via the Japan Proteome Standard Repository[26] with the dataset identifier JPST000395.

A total of 1960 and 1945 proteins were identified in *H. volcanii* and *N. magadii* (Table S1), respectively, which accounts for almost 50% of the complete predicted proteome of each haloarchaeon. Furthermore, approximately 30% of the predicted integral membrane proteins (one or more TMS) were identified for both haloarchaea. Figure 1A shows the distribution of all identified proteins taking into account both microorganisms and growth stages. On the other hand, Figure 1B summarizes the number of significantly regulated proteins. The statistical analysis indicates that 532 proteins from *H. volcanii* and 432 proteins from *N. magadii* have changed significantly between EXP and ST phase (Table S2). Considering the predicted functions described in the UniProt database (The UniProt Consortium, 2017), the differential proteins were grouped into a modified version of the broad functional categories proposed by Makarova et al[27] (Figure 1C).

Both haloarchaea showed a significant increase in many high affinity ABC-type transporter components during entrance into ST phase (51 in *H. volcanii* and 28 in *N. magadii*, Table S2). Most of them were predicted to transport peptides (e.g. Nmag_0184/HVO_0062, *dppA1*) and metal ions (e.g. Nmag_2678/HVO_0891, *nosF*). Transporters for sugars were also accumulated in the ST phase in *H. volcanii* (e.g. HVO_B0106, *tsgA8*; HVO_2113, *tsgA2*) while a homologue to the bacterial carbon starvation protein CstA (involved in the starvation response and peptide uptake in bacteria) showed

a substantial increase (16 fold) in *N. magadii* (Nmag_0850).[28, 29] An overall induction of nutrient scavenging systems is not surprising given that "food" restriction predominates in the ST phase.

Other affected transporters included the mechanosensitive ion channel proteins MscS (HVO_1165, HVO_1659) and potassium ion transmembrane transporters TrkA (e.g. HVO_2617, Nmag_2258) which are particularly important in halophiles since they provide protection against hypo-osmotic shock. A stomatin family protein was notoriously up-regulated in both organisms (Nmag_0657, 54 fold/HVO_0035, 12 fold). Members of this family of membrane proteins have been shown to modulate ion channel activity in eukaryotes.[30] Whether this role is conserved in archaea remains to be investigated.

Not surprisingly, homologues of the universal stress protein UspA (e.g. Nmag_1040, HVO_1481) increased in amount upon entrance into ST phase. This family is conserved in eukaryotes, bacteria and archaea and is important for survival during cellular growth arrest in *E. coli*.[31] In addition, in *H. volcanii*, proteins involved in the biogenesis of the pili, such as pilins PilA1, PilA6, PilB3, PilC3 and prepilin/preflagellin peptidase PibD were also up-regulated. PilA6, related to the stimulation of microcolony formation,[32] was especially up-regulated (23 fold). In archaea, type IV pili-like structures facilitate cell–cell associations in early stages of biofilm formation and in the uptake of extracellular substrates, including a variety of nutrients.[33] Altogether, these results suggest that developing a general stress resistance and increasing the uptake of nutrients is central for the survival of haloarchaea in the ST phase.

The amount of several proteins involved in the catabolism of fatty acids (β-oxidation) was also incremented in ST phase in both haloarchaea (Table S2), mainly acyl-CoA synthetases (e.g. HVO_A0156, Nmag_2120), acyl-CoA dehydrogenases (e.g. HVO_1140, Nmag_3134), enoyl-CoA hydratases (e.g. Nmag_1031, HVO_A0525) and 3-hydroxyacyl-CoA dehydrogenases (e.g. HVO_A0524, Nmag_1034). In contrast to bacteria and eukaryotic cells, the phospholipids of archaeal membranes are built with isoprenoids,[34] however, fatty acids can be found associated to proteins

such as energy-transducing membrane complexes.[35] The overall up-regulation of these enzymes suggests that fatty acids may be used as an alternative carbon source during starvation. This type of adaptation has been reported for bacteria [36] and eukaryotes.[37]

Several peptidases showed an increment in the EXP-ST phase transition in *H .volcanii* and *N. magadii*. These include family protein M24 (HVO_A0535/Nmag_3901), M28 (HVO_0836, Nmag_3998), M42 (HVO_2759) and HtpX-like protease (HVO_2904). HtpX belongs to the heat shock protein (Hsp) family, and has a central role (in conjunction with ATP-dependent protease FtsH) in the quality control of membrane proteins in *E. coli* under stress.[38] The up-regulation in peptidases/proteases not only insures the degradation of abortive/misfolded proteins, but it also facilitates *de novo* protein synthesis during nutrient limitation by increasing the amino acid pool. This correlates with the fact that most proteins involved in amino acid biosynthesis were less-represented in ST phase in both strains (e.g. methionine synthase II, Nmag_1090/HVO_2742; glutamate synthase large subunit, HVO_0869). On the other hand, those enzymes participating in amino acid catabolism were over-represented (e.g. HVO_A0559, histidine ammonia-lyase). In *N. magadii,* 1,2-phenylacetyl-CoA epoxidase, involved in the catabolism of phenylacetate,[39] showed a major increase with fold changes of 18, 80 and 495 (subunits A, B and C, respectively), suggesting a possible role of this aromatic compound as an alternative growth substrate. Considering that organisms capable of degrading aromatic hydrocarbons are of particular relevance for bioremediation,[3] these results suggest *N. magadii* could be a potential candidate for the bioremediation of contaminated hypersaline and alkaline environments.

On the other hand, an overall decrease was observed for proteins involved in genetic processes such as transcription, translation, RNA maturation, DNA replication/repair (78 and 57 down-regulated proteins in *H. volcanii* and *N. magadii*, respectively). These included 31 and 26 ribosomal proteins in *H. volcanii* and *N. magadii,* respectively (from approximately 50 detected), translation initiation/elongation factors (4 out of the 14 found in *H. volcanii* and 7 from the 13

detected in *N. magadii*), replication protein A, UvrABC system protein A, among others. A decrease in five subunits of the RNA polymerase (A`, A``, B`, B``, D) was observed only in *H. volcanii*. Cell division protein FtsZ type II (Nmag_0980, HVO_0581) also decreased 4-fold in both organisms. An overall down-regulation of the mechanisms of DNA conservation and gene expression in the ST phase was not surprising as it has been reported in bacteria[40] and haloarchaea.[7, 18]

Several proteins involved in signal transduction and gene regulation were affected, such as IclR family transcription regulators (e.g HVO_2130, Nmag_0813), Lrp/AsnC family transcription regulators (HVO_0240, HVO_2029, HVO_2507) and histidine kinases (HVO_0570, HVO_1397), among others. The transcription regulator families Lrp (leucine-responsive regulatory protein) and IclR (Isocitrate lyase regulator) are the major regulators of genes involved in processes as different as transport of nutrients, pili synthesis, biosynthesis of amino acids, catabolism, and the use of various carbon sources in bacteria and archaea.[41, 42] Taking into account the global changes detected in the EXP-ST phase transition, it is likely that these transcription regulators control many of these cellular processes by modulating protein expression.

In conclusion, using label-free shotgun comparative proteomics we have identified proteins with differential abundance in the haloarchaea *H. volcanii* and *N. magadii* in the EXP-ST growth phase transition. Overall, both haloarchaea showed similar adaptations in response to this transition, including an increase in high affinity-transporters, stress proteins, peptidases, catabolic enzymes as well as a general decrease in proteins involved in genetic processes and changes in transcriptional regulators. This study provides a platform of data that complements the current knowledge on ST phase responses in bacteria and specifically extents the information to archaeal cells and extremophiles. It also reports the first whole cell proteome analysis of the alkaliphilic archaeon *N. magadii.*

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Conflict of Interest

The authors have declared no conflict of interest.

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Figure 1. Comparison of identified and differential proteins in EXP and ST phase from *H. volcanii* and *N. magadii*. A. Venn diagram representing the number of all identified proteins in both growth stages. **B.** Number of significantly differential proteins between EXP and ST phase. Proteins were considered as significantly regulated at a log2 value of at least 1 and -1 (2 fold change) and an FDR (q-value) < 0.05. **C.** Classification of significantly up/down-regulated proteins in ST phase into functional categories.